EXHIBIT A

2/15/00

~~Clean DNA first.~~

~~Run a gel~~

~~there are just a~~

~~little DNA in expect size~~

~~So. improve Digest~~

Digest

DNA 40 ul buffer 10 ul
H_2O 42.5 ul EcoRI 8.5 ul } overnight 37°C

[illegible] GENOSYS HAPDAMP[illegible] 5'-[illegible]
[illegible] GENOSYS HAPDAMP2 5'-TGTGCCGTTTGGTACCTATA
3 [illegible] GENOSYS HAPDAMP2 5'-TGTGCCGTTTGGTACCTATA

→ primer → New

Digest – to ~~check~~ differential digest procedure for dam gene detection 2/17/00

11039 or D153 DNA 1 ul

10 x buffer 1 ul

H_2O 7.5 ul

enzyme 0.5 ul

↓

3 digest Sau 3A I Dpn I Mbo I

Sau 3A I ↓ both

Dpn I: unmythlased gene

Mbo I ↓ do-mythlased gene

Digest 37°C 4 hr.

Signed

Read and Understood By

[signature]

Signed

Inverse PCR procedure

Use 100 ng EcoRI digest set up 3 ligations

1039 EcoRI cut DNA	1	2	5
10× buffer	5	5	5
T4 ligase (NEB)	0.5	0.5	0.5
H_2O	33.5	33.5	33.5
	50 ml		

16°C overnight ; heat inactivate

for PCR

2+1 100

Run a gel to detect differential digest procedure for dam gene.

Ladder ① 1039 Sau3AI ③ 1039 MboI ④ 1039 DpnI

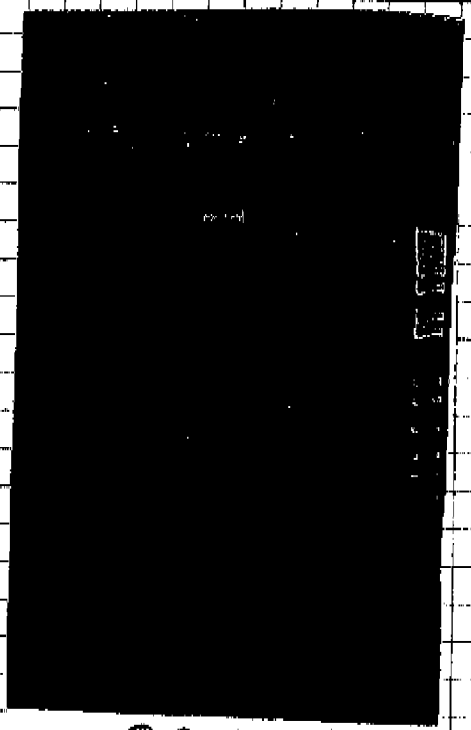
② D153 ⑤ D153 ⑥ D153

there once some is dam gene in D153.

Sau3AI → digest GATC both mathyled and unm...

MboI → digest GATC only un....

DpnI → digest GATC only mathylated.



⑥ ⑤ ④ ③ ② ①

Read and Understood By

Signed